

WHAT IS CLAIMED IS:

1. 1. A method for enzymatically processing nucleic acid molecules in a mixture that includes a chaotropic agent, degraded and denatured proteins and RNA and DNA molecules freed of bound proteins, comprising diluting said mixture with at least one aqueous reagent to reduce the concentration of chaotropic agent to less than 0.05 M and subjecting at least some of said RNA and DNA molecules to at least one enzymatic process without removal of or isolation of said RNA and DNA molecules from each other or from the other components of the reaction mixture.
1. 2. The method according to claim 1, wherein the mixture is diluted to reduce the concentration of chaotropic agent to less than 0.01M.
1. 3. The method according to claim 1 or claim 2 wherein said mixture includes at least one component selected from the group consisting of a cell-lysing detergent, a reducing agent, a water-miscible solvent, a chelating agent, and a neutralizing buffer.
1. 4. The method according to any of claim 1 to claim 3, wherein dilution is accomplished by serial addition of at least two aqueous reagents.
1. 5. The method according to any of claim 1 to claim 4 wherein said at least one enzymatic process includes exponential nucleic acid amplification.
1. 6. The method according to claim 5 wherein said amplification is a polymerase chain reaction process.
1. 7. The method according to claim 5 wherein said at least one enzymatic process includes reverse transcription.
1. 8. The method according to any of claim 1 to claim 7 wherein an enzyme for performing said at least one enzymatic process is included in said at least one aqueous reagent.
1. 9. The method according to claim 8 wherein said enzyme is selected from the group consisting of a DNase, a reverse transcriptase, a DNA polymerase, and a glycosidase.

- 1 10. The method according to claim 8 wherein the dilution and the at least one enzymatic
2 process are performed in the same container.
- 1 11. The method according to claim 10 wherein the container is selected from the group
2 consisting of a tube, a microtiter plate and a microfluidic device.
- 1 12. The method according to any of claim 1 to claim 11 wherein said mixture is prepared by
2 incubating a sample containing protein-bound RNA and DNA molecules and a chaotropic
3 agent-containing disruption reagent at a concentration of chaotropic agent of at least
4 about 2 M.
- 1 13. The method according to claim 12 wherein incubation of the sample and disruption
2 reagent includes heating, whereby chaotropic agent is concentrated.
- 1 14. The method according to claim 13 wherein said heating is sufficient such that the
2 resulting mixture is at least semi-dry.
- 1 15. The method according to claim 14 wherein the resulting mixture is stored prior to use.
- 1 16. The method of any of claim 12 to claim 14 wherein incubating and dilution are carried
2 out in the same container.
- 1 17. The method according to any of claim 12 to claim 16 wherein said disruption reagent is a
2 dry reagent.
- 1 18. The method according to claim 19 wherein said dry disruption reagent is prepared from a
2 mixture that includes a water-miscible solvent.
- 1 19. The method according to claim 17 or claim 18 wherein said dry disruption reagent is
2 prepared from a mixture that includes at least one component selected from the group
3 consisting of a cell-lysing detergent, a reducing agent, a chelating agent and a
4 neutralizing buffer.
- 1 20. The method according to any of claim 17 to claim 19 wherein said dry reagent is adhered
2 to a surface of a container..

- 1 21. The method according to claim 20 wherein said surface is the inner surface of a tube, a
2 tube cap, a wall of a microtiter plate or a microtiter plate cover.
- 1 22. The method according to any of claim 12 to claim 21 wherein said sample comprises
2 from a fraction of a cell up to 200 cells.
- 1 23. The method according to any of claim 12 to claim 22 wherein the amount of disruption
2 reagent is sufficient to provide a 2 M-8 M concentration of the chaotropic agent in a
3 volume of 20 to 50 nl.
- 1 24. A device for use in processing a biological sample containing protein-bound RNA and
2 DNA molecules to free said molecules from proteins comprising a dried disruption
3 reagent including a chaotropic agent adhered to a surface of a container or container part.
- 1 25. The device according to claim 24, wherein said container or container part is selected
2 from the group of a tube, a tube cap, a microtiter plate and a cover for at least one well of
3 a microtiter plate.
- 1 26. The device according to claim 24 or claim 25, wherein said container or container part
2 contains multiple chambers connected by flow channels.
- 1 27. The device according to any of claim 24 to claim 26, wherein said dried disruption
2 reagent contains an amount of chaotropic agent that will produce a concentration of 2 M
3 – 8 M chaotropic agent when dissolved in 20 – 50 nl of water.
- 1 28. The device according to any of claim 24 to claim 27, wherein said dried disruption
2 reagent contains a detergent.
- 1 29. The device according to any of claim 24 to claim 28, wherein said dried disruption
2 reagent includes at least one component from the group of a reducing agent, a chelating
3 agent, a water-miscible solvent and a buffer.
- 1 30. A kit comprising at least one reagent useful for enzymatic processing of nucleic acids and
2 a device according to any of claim 24 to claim 29.
- 1 31. The kit according to claim 30 including primers and enzyme for reverse transcription.

- 1 32. The kit according to claim 31, including a DNase enzyme.
- 1 33. The kit according to any of claim 30 to claim 32, including nucleic acid amplification
2 reagents comprising at least DNA polymerase and amplification buffer.
- 1 34. The kit according to claim 33, including at least one pair of polymerase chain reaction
2 primers and at least one sequencing primer.
- 3 35. The kit according to claim 33, including at least detection reagent.
- 1 36. The kit according to claim 35, including at least one dual labeled fluorescent
2 hybridization probe.